

(12)

LEVEL II

AD

ADA 083995

STUDIES ON TROMBICULID MITES (CHIGGERS) AND
OTHER ECTOPARASITES AS VECTORS OF RICKETTSIAL
INFECTIONS
FINAL SCIENTIFIC REPORT

by

Charles L. Wisseman, Jr., M.D.

March 1979
(For the period 1 Sept 1969 to 30 June 1977)

Supported by

U.S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND
Office of the Surgeon General, Washington, D.C. 20310
in cooperation with the Commission on Rickettsial Diseases
of the Armed Forces Epidemiological Board

Contract No. DADA17-70-C-0047
University of Maryland School of Medicine
Department of Microbiology
Baltimore, Maryland 21201

see contract ed
4/12/79

DTIC
ELECTE
MAY 7 1980

S B D

DOD DISTRIBUTION STATEMENT

Approved for public release;
distribution unlimited

The findings in this report are not to be construed as an official Department
of the Army position unless so designated by other authorized documents.

80 . 4 28 080

DDC FILE COPY.

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM	
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER	
	AD-A083 995		DIV
4. TITLE (and Subtitle)	5. TYPE OF REPORT & PERIOD COVERED		
6. STUDIES ON TROMBICULID MITES (CHIGGERS) AND OTHER ECTOPARASITES AS VECTORS OF RICKETTSIAL INFECTIONS.	Final rept. 1 Sep 1969 - 30 June 1977		
7. AUTHOR(s)	8. CONTRACT OR GRANT NUMBER(s)	9. PERFORMING ORG. REPORT NUMBER	
10. Charles L. Wisseman, Jr.	15. DADA17-70-C-0047		
11. PERFORMING ORGANIZATION NAME AND ADDRESS	12. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS		
University of Maryland School of Medicine Baltimore, Maryland 21201 <i>Dept of microbiology</i>	621104/3A1621104831		
13. CONTROLLING OFFICE NAME AND ADDRESS	14. REPORT DATE	15. NUMBER OF PAGES	
US Army Medical Research and Development Command Fort Detrick Frederick, Maryland 21701	Mar 1979	20	
16. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	17. SECURITY CLASS. (of this report)	18. DECLASSIFICATION/DOWNGRADING SCHEDULE	
12/19	Unclassified		
19. DISTRIBUTION STATEMENT (of this Report)			
Approved for public release; distribution unlimited.			
20. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)			
21. SUPPLEMENTARY NOTES			
22. KEY WORDS (Continue on reverse side if necessary and identify by block number)			
scrub typhus, chigger-borne rickettsiosis chiggers, trombiculid mites, vectors, rickettsial infections			
23. ABSTRACT (Continue on reverse side if necessary and identify by block number)			
<p>Research was carried out primarily on the ecology of chigger-borne rickettsiosis (scrub typhus) and the classification of trombiculid mites (chiggers). A total of 27 articles were published with the support of this contract and its predecessors. Included was a critical and extensive review of the ecology of scrub typhus. The demonstration that chiggers belonging to the vector-group of <i>Leptotrombidium</i> occur in the mountains of Ethiopia suggests that scrub typhus may exist there, although it has not yet been conclusively shown to be endemic anywhere in Africa.</p>			

100808

In the course of the investigations, data were obtained supporting the concept that chiggers are the actual reservoirs as well as the vectors of chigger-borne rickettsiosis. Thus, the infection is maintained in nature by transovarial transmission of rickettsiae from mother to offspring. However, the studies also indicate that under certain conditions (e.g., reattachment, or occasional acquisition of true infection), chiggers acquiring Rickettsia tsutsugamushi by feeding on rickettsemic hosts, may actually play a role in perpetuating the cycle or infecting another mammal. As emphasized in some of the articles published under these auspices, topography, geography and ecology are each insufficient to determine the endemicity of scrub typhus in any particular area in the continents where the rickettsiosis is known to occur. Factors like geological history, "ecological islands," and the nature of the fauna must also be considered. Another important factor is the sequential ecological succession that occurs with the passage of years after the terrain has been modified by man or nature, and whereby the kind of local rats and chiggers vary accordingly.

Under this contract and its predecessors, a total of 62 new species of chiggers were described. The more recent papers included a revisionary one on Helenicula, a major parasite of rats.

TABLE OF CONTENTS

1. Foreward	iii
2. Summary of Final Report	iv
3. Final Report	1
I. Introduction	1
II. The Ecology of Chigger-Borne Rickettsiosis	1
A. Background	1
B. The Acquisition of <u>R. tsutsugamushi</u> by Chiggers During the Feeding Process	4
C. The Possible Occurrence of Chigger-Borne Rickettsiosis in Africa.....	5
D. The "Zoonotic Tetrad" of Chigger-Borne Rickettsiosis ...	5
E. Topography & Endemicity of Chigger-Borne Rickettsiosis .	6
F. Other Studies on Chigger-Borne Rickettsiosis	7
G. Histological Studies on Chigger Attachment	8
III. Techniques of Rearing Chiggers	8
IV. Taxonomy	8
V. Other Relevant Studies	9
VI. Bibliography & References Cited	10
4. Personnel	13

DTIC
ELECTE
S MAY 7 1980 **D**
B

ACCESSION for		
NTIS	White Section	<input checked="" type="checkbox"/>
DDC	Buff Section	<input type="checkbox"/>
UNANNOUNCED		<input type="checkbox"/>
JUSTIFICATION		
BY		
DISTRIBUTION/AVAILABILITY CODES		
Dist.	AVAIL	and/or SPECIAL
A		

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities & Care," as promulgated by the Committee on the Guide for Laboratory Resources, National Academy of Sciences - National Research Council.

SUMMARY

Under this Contract, research was carried out primarily on 1) the ecology of chigger-borne rickettsiosis (scrub typhus); 2) the classification of trombiculid mites (chiggers), including known and potential vectors of this infection; 3) corollary subjects which were by-products of the above studies and 4) development of pertinent techniques. Of the 27 articles published with the support of this Contract, 13 deal principally with the ecology of chigger-borne rickettsiosis; 4 with vectorship of that infection; 4 with taxonomy and 6 papers treat other ectoparasites or their hosts. One of the publications is an extensive and critical review of the ecology of scrub typhus and includes new and original ideas on the subject. This rickettsiosis is considered to be intimately associated with wild forms of Rattus (Rattus) and chiggers of the Leptotrombidium deliense complex. Both ecologically and geographically it is restricted to areas where those particular mammals and mites co-exist. Chigger-borne rickettsiosis is not expected to occur west of Eastern Iran, or in the New World, etc. or in most of the primary forest in S.E. Asia. Our findings of such chiggers in the mountains of Ethiopia re-opens the question as to whether scrub typhus occurs in Africa.

In the course of the investigations, data were obtained supporting the concept that chiggers are the actual reservoirs as well as the vectors of chigger-borne rickettsiosis. Thus, the infection is maintained in nature by transovarial transmission of rickettsiae from mother to offspring. However, the studies also indicate that under certain conditions (e.g., reattachment, or occasional acquisition of true infection), chiggers acquiring Rickettsia tsutsugamushi by feeding on rickettsemic hosts, may actually play a role in perpetuating the cycle or infecting another mammal. As emphasized in some of the articles published under these auspices, topography, geography and ecology are each insufficient to determine the endemicity of scrub typhus in any particular area in the continents where the rickettsiosis is known to occur. Factors like geological history, "ecological islands," and the nature of the fauna must also be considered. Another important factor is the sequential ecological succession that occurs with the passage of years after the terrain has been modified by man or nature, and whereby the kind of local rats and chiggers vary accordingly.

The mass cultures of trombiculid mites prepared in this program were unique, and as many as could be handled by the Army were supplied for future maintenance. The successful techniques are being described in an article in preparation.

Under this Contract and its predecessors, a total of 62 new species of chiggers were described. The more recent papers included a revisionary one on Helenicula, a major parasite of rats.

C.L. WISSEMAN, JR., M.D., PRINC. INVEST.: DA49-70-C-0047 "TRONBICULID MITES (CHIGGERS) AND OTHER
ECTOPARASITES AS VECTORS OF RICKETTSIAL INFECTION." (1 SEPT. 1969-31 DEC. 1975) FINAL REPORT

I. INTRODUCTION

In essence, Contract No C-0047 was a continuation of the acarological aspects of Contracts DA49-193-MD-2074, and DA49-193-MD-2277 (which is still on-going and is concerned with rickettsial research), and hence this Final Report refers in parts to results obtained based on the earlier studies and published during the tenure of Contract C-0047. It should also be noted that at the onset of this Contract period, we were directed by the Army authorities to cease all taxonomic work forthwith and restrict our investigations solely to experimental studies on scrub typhus (chigger-borne rickettsiosis), whereas later, in the middle period, we were summarily told to do just the opposite, i.e., kill off the colonies of chiggers we were using in experiments and solely undertake taxonomic investigations. In view of the sudden and complete changes of direction, the long-range objectives could not be fully achieved, and we still are working on some of the manuscripts.

In the bibliographic citations in this Report, the designations after the reference number indicate the following:

** pertains to work specifically done under this Contract, and by the scientists listed herein.

* indicates studies accomplished with the partial support of the Contract, by us or our colleagues.

refers to other articles published under the auspices of the Contract.

© signifies other Army-sponsored research, e.g. previous Contract, undertaken by us.

Unmarked citations refer to work by other investigators.

II. THE ECOLOGY OF CHIGGER-BORNE RICKETTSIOSIS.

A. BACKGROUND

1. In 1974 we published an extensive and critical review of the ecology of chigger-borne rickettsiosis (30**). This presented a synthesis and analysis of available information, including new and original material based upon work undertaken in this Contract and its predecessors, as well as observations from the general literature. As background for some of the points made below, we cite the conclusions reported in that paper, viz.

"(1) The characteristic epidemiological features of chigger-borne typhus, such as a marked focal distribution of cases occurring as a sharp outbreak in terrain of secondary vegetation, etc., may all be explained in terms of the attributes of the vector chiggers.

(2) The rickettsiosis exists in a wide variety of habitats, ranging from semideserts to alpine meadows and subarctic scree in the Himalayas, and from disturbed rain forest to seashores. The endemicity in modified dipterocarp forest may be comparable to that in the lalang on the periphery, depending upon time and place. The name scrub typhus hence is a misnomer and is misleading regarding the type of endemic habitats that may be hazardous.

(3) All known foci are characterized by changing environmental conditions, whether induced by man or nature as a sudden event (e.g., clearing of forests, landslides), or gradual (changes along untouched fringe habitats) or cyclical (tidal zones, spring floods,

glacial runoff).

(4) Chiggers of the Lentotrombidium deliense-group are the main vectors to man and perhaps to other hosts as well. So far as known, these chiggers are abundant wherever this infection has been reported.

(5) Rats, particularly wild rats of the subgenus Rattus, are found in all known endemic areas, and they serve as prime hosts of Lentotrombidium throughout their native ranges.

(6) Some sort of transitional or secondary vegetation is characteristic of all terrain where outbreaks have occurred, and is present in some form in all known foci of chigger-borne rickettsiosis, even if only a fringe habitat along streams in deep forest.

(7) The conjunction of the L. deliense-group, wild rats and transitional vegetation (such as grasses, herbs, secondary-growth trees and shrubs) is so intimately connected with this infection that, together with R. tsutsugamushi, these factors are regarded as constituting the "zoonotic tetrad of chigger-borne rickettsiosis" and are believed to have evolved together as a bieme.

(8) It is believed that as Rattus and associated members of the subgenus Lento-
trombidium penetrated new areas and habitats during the peregrinations of these rodents through the eons, they gave rise to new taxa in the course of evolution. Many of these became direct participants in the cycles of this rickettsiosis, but as the chiggers adapted to other kinds of hosts living in the paths of the emigrating rats, such mammals (and perhaps their own trombiculid fauna) became secondarily involved in the ecology of the infection (e.g., voles in the Himalayas and Japan) and served to extend its range.

(9) The role of other kinds of chiggers is unclear, but members of Neotrombicula, Gehrlienia, etc., may prove to serve as minor vectors to man, or be important as intraspecific vectors.

(10) Naturally infected chiggers presumably constitute the main reservoirs of R. tsutsugamushi in nature, maintaining and perpetuating the cycle of infection by transovarian transmission of rickettsiae from mother to progeny, especially in certain family lines of vector species.

(11) Natural infection with R. tsutsugamushi is expected to occur in any mammal and presumably, bird whose habits bring them into contact with ground infested with vector species of chiggers in endemic areas.

(12) While natural infection is widespread in a variety of theraphions, especially ground-infesting species, it may be that rickettsiae from such hosts do not regularly enter the epidemiological picture. Thus, chiggers normally feed but once as parasites and hence any acquired infection will not be passed to a 2nd host unless transovarian transmission to the next generation is effected, or unless the chigger is exceptional and not only will reattach to another host but actually succeeds in transmitting infection. The laboratory data to date indicate that these events are unusual, but even so, if they also occur in nature, they may be significant in the ecology of this rickettsiosis because the enormous numbers of chiggers involved may compensate for the low rate of occurrence. Transovarian transmission of acquired infection, even if a rare event, could result in an increased number of family lines of infected chiggers, provided succeeding generations continued to effectively transmit the infection transovarially.

(13) Even if they do not constitute true reservoirs of infection, rats, field mice, voles, shrews, ground squirrels and tree shrews which come in contact with the ground in endemic foci are nevertheless important in the ecology of chigger-borne rickettsiosis in that they serve as important hosts of the L. deliense-complex, which thrive in such habitats.

C.L. WISSEMAN, JR., M.D., PRINC. INVEST.: DADA17-70-C-0047 "TROMBICULID MITES (CHIGGERS) AND OTHER
ECTOPARASITES AS VECTORS OF RICKETTSIAL INFECTION," (1 SEPT. 1969-31 DEC. 1975) FINAL REPORT

(14) Commensal rats and arboreal animals play little or no part in the ecology and epidemiology of this rickettsiosis, presumably because their habitats and haunts generally preclude infestation with Leptotrombidium and interfere with the proper post-larval development of such chiggers if they do become infested.

(15) Just as new endemic foci may appear when the ecological features of a habitat are modified and members of the zoonotic tetrad become predominant in consequence, so may endemicity in existing areas wax or wane as local conditions affect the rodent and chigger fauna and the flora, with the passage of time. Several adjacent foci of seemingly similar appearance, or even different sections of a "homogeneous" biotope, may thus vary considerably with respect to the particular phase of the cycle of chigger-borne rickettsiosis. For example, one portion may no longer be hyperendemic, or the components and proportions of the chigger or rat population may differ because of this time factor.

(16) The available but limited data suggest that there is no true sylvan cycle of chigger-borne rickettsiosis in the strictly primary forest which deeply involves indigenous "white Leptotrombidium" and Gabrielis, etc., and rats of the subgenera Lenothrix, Maxomys, Stenomys, etc. However, members of the L. deliense-complex and the subgenus Rattus do occur in restricted suitable fringe habitats deep in the forest, e.g., along game tracks and by cliffs, and these may become numerous when conditions are modified, as when trees are felled and secondary vegetation appears on a larger scale or in new pockets. Hyperendemic foci may soon follow, and R. tsutsugamushi infection may become common in R. (Maxomys) and R. (Lenothrix) and other "jungle rats" as well.

(17) Areas with utterly different topographic features, well separated by barriers like deserts and mountain massifs, may harbor "ecological islands" and "oases" with a relict population sharing major elements of the rodent and ectoparasite fauna and associated infections such as chigger-borne rickettsiosis. These reflect conditions of the geological past.

(18) Rickettsia tsutsugamushi is significantly different from other members of the genus Rickettsia, and the similarities between them probably represent convergence towards an existence as intracellular parasites with cycles in both mammals and arthropods. On the other hand, the so-called serotypes of R. tsutsugamushi represent divergent forms.

(19) The widespread distribution of certain strains, and the fact that some serotypes may be transmitted by several species of Leptotrombidium lend support to the belief that infection acquired from theraphions may be an important factor in the cycles of R. tsutsugamushi in nature. However, there remains a great deal to be learned about the properties of R. tsutsugamushi and their connection with the ecology of this rickettsiosis.

(20) Faunal surveys, utilizing as indicators the concurrent presence of the L. deliense-group and wild Rattus (or theraphions already implicated elsewhere) are of value in predicting whether chigger-borne rickettsiosis is endemic in locality or habitat. On this basis, it is anticipated that this infection exists in unrecognized form in Turkmeniya, northeastern Iran, parts of Soviet Central Asia and other areas bordering the known endemic regions. Cryptozootic infection, or else enzootic infection coupled with an occasional human case, is expected in the peripheral areas of this rickettsiosis, as seen in Korea, Hokkaido, W. Pakistan, Tadzhikistan, etc.

(21) Chigger-borne rickettsiosis is absent from the New World, northern USSR and Europe. More information is needed before its status in Africa and the Middle East can be clarified."

C.L. WISSEMAN JR., M.D., PRINC. INVEST.: DADA17-70-C-0047 "TROMBICULID MITES (CHIGGERS) AND OTHER
ECTOPARASITES AS VECTORS OF RICKETTSIAL INFECTION." (1 SEPT. 1969-31 DEC. 1975) FINAL REPORT

2. Additional points about the ecology of chigger-borne rickettsiosis were presented in some of our other articles (30**, 33**, 31*).

B. STUDIES OF THE ACQUISITION OF R. TSUTSUGAMUSHI BY CHIGGERS DURING THE FEEDING PROCESS.

1. Much of the work done under this Contract and its predecessors was concerned with the fascinating questions as to whether chiggers could acquire Rickettsia tsutsugamushi while feeding, become truly infected, and transmit the infection to the next generation. If so, rodents, as well as naturally-infected lines of chiggers could serve as true reservoirs of chigger-borne rickettsiosis. The results of our studies with uninfected lines of vector-species (Leptotrombidium deliense, L. arnica, L. fletcheri etc.) and suspected vectors (Gahriopsis (S.) ligula) may be summarized as follows, as per our 1975 article on the subject (33**).

1) "Although vector chiggers undoubtedly are important true reservoirs of chigger-borne rickettsiosis, the following suggests that theraphions may also serve as a wellspring for maintenance of the rickettsial cycle in nature."

2) "Uninfected chiggers may acquire rickettsiae while feeding on hosts with rickettsemia, and such ingested organisms may at least survive for weeks. Transovarian transmission to the next generation was demonstrated in one pool of chiggers with such acquired rickettsiae. An appreciable number of chiggers may detach from their original host, particularly if it dies prematurely, and successfully attach to a second host and develop normally thereafter. In one instance, the chiggers that detached from the second host still bore evidence of rickettsiae acquired by feeding on the first one, more than one week earlier. Natural infection with R. tsutsugamushi is often common in a variety of ground-dwelling theraphions in endemic areas. Vector species may be extremely abundant on such hosts. The geographic and ecologic distribution and host relationships of known serotypes of R. tsutsugamushi do not correlate with those of the vector species of Leptotrombidium. There are conflicting laboratory data on the efficiency of the mechanism of transovarian transmission, even when the tests are limited to using susceptible hosts to feed naturally infected chiggers. There is no information available as to the source of natural infection in generations of chiggers in the field, where there are many complicating factors; for example, both immune and susceptible hosts may be infested with large numbers of chiggers in various stages of engorgement, representing several species of Leptotrombidium, and including both infected and uninfected individuals, with the possibility that more than one serotype of R. tsutsugamushi is present in the pools of chiggers. There may be marked genetic variability in chiggers with respect to the ability to acquire rickettsiae (and infection) by feeding. Similarly, there probably are significant differences in family lines of chiggers regarding the virulence of the R. tsutsugamushi they carry and in the efficiency of the mechanism of transovarian transmission."

3) "More data are required on most of these points before the existing questions can be answered about the importance of theraphions as a source of rickettsiae to chiggers."

C.L. WISSEMAN JR., M.D., PRINC. INVEST.: DADA17-70-C-0047 "TRONBICULID MITES (CHIGGERS) AND OTHER
ECTOPARASITES AS VECTORS OF RICKETTSIAL INFECTION," (1 SEPT. 1969-31 DEC. 1975) FINAL REPORT

C. THE POSSIBLE OCCURENCE OF CHIGGER-BORNE RICKETTSIOSIS IN AFRICA.

1. An important but unresolved question is whether this rickettsiosis exists in Africa. There have been various reports claiming endemicity, but since they were based upon serology of dubious validity, or purely upon clinical symptoms, they have not received acceptance (30**). One argument advanced against such claims has been that no true Leptotrombidium (Leptotrombidium) chiggers had ever been found in Africa. However, in serological studies supported by this Contract and based upon travel accomplished with funds provided by the Office of Naval Research regarding murine typhus, we collected two species of Leptotrombidium in Ethiopia, both new to Science, and one of them is a true member of the subgenus Leptotrombidium, akin to the L. deliense-complex of classical vectors. The latter new species is allied to suspected vectors of chigger-borne rickettsiosis in endemic foci we had discovered in the Himalayas in our Army-sponsored projects (vide 30**, 358, 278, 288). Inasmuch as it has been possible to demonstrate endemicity of R. tsutsugamushi wherever the tests were undertaken in the presence of members of the L. deliense-complex (30**), there now is a more justifiable rationale for the belief that chigger-borne rickettsiosis exists in Africa.

2. The new Ethiopian Leptotrombidium (Leptotrombidium) was collected in the mountains near Ankoher, at 11,000 ft. elevation, from a variety of murine genera, including rats that formerly had been placed in the genus Rattus. (As we have stressed (30**) both scrub typhus and the subgenus Leptotrombidium are intimately associated with the genus Rattus.) Interestingly enough, the Pakistan species which we described and which this new one resembles, is also a high-altitude form (258).

3. Notably, the fleas and rodents of the Ankoher region are closely related to the fauna of the mountains of southern Ethiopia and those of Uganda, Kenya, etc., the intervening vast areas of semidesert or low-lying rain-forest notwithstanding. It appears that these isolated montane areas are "ecological islands" representing former eons when the overall climate must have been colder, and the lower elevations covered with different types of vegetation, than now. The situation seems analogous to what we have hypothesized for the isolated mountains of southwest Asia (358, 248, 288, 30**). It seems likely that "endemic" ectoparasite-borne infections found in one area likewise occur in the others, as we showed was the case with tick-borne rickettsiosis in Pakistan (158, 168).

4. The second new Leptotrombidium belongs to a different subgenus and comes from the semi-arid acacia groves of the Rift Valley, an entirely different habitat of a type found throughout much of Africa, and with a rodent and ectoparasite fauna that have only a few elements in common with those of the montane areas.

D. THE "ZONOTIC TETRAD" OF CHIGGER-BORNE RICKETTSIOSIS

1. We have stressed that in all known endemic foci of this rickettsiosis there is an invariable concurrence of four factors: 1) chiggers of the subgenus Leptotrombidium; 2) wild rats, especially of the subgenus Rattus; 3) secondary vegetative growth, in areas of changing conditions; and 4) the etiological agent, R. tsutsugamushi within the Leptotrombidium (30**). It was also postulated as corollaries that the subgenus Leptotrombidium and wild

C.L. WISSEMAN JR., MD., PRINC. INVEST.:DADA17-70-C-0047 "TROMBICULID MITES (CHIGGERS) AND OTHER ROTOPARASITES AS VECTORS OF RICKETTSIAL INFECTION," (1. SEPT. 1969-31 DEC. 1975) FINAL REPORT

forms of Rattus (Rattus) were recently evolved forms which were so intimately associated with secondary vegetation that these chiggers and rats occurred only in such habitats, and hence would not be found in true primary jungle (a much older habitat, with its own fauna and flora). We have pointed out that where such chiggers, rats or this infection had been reported in "primary forest," the investigators in question were actually referring to areas of disturbed or secondary forest that were deep within "primary jungle," near settlements of aborigines, or else were "fringe habitats," as exist along streams and tracks. The point is a fundamental one regarding evolution, zoogeography and the distribution of related infections, and we have been seeking data on this score in New Guinea, since virtually all accessible forested areas in S.E. Asia have been modified by man. We have also expressed the opinion that the subgenus Leptotrombidium arose in S.E. Asia and penetrated the peripheral areas, including New Guinea, only relatively recently. The data from New Guinea support these various contentions.

2. Data have been obtained for two forested areas from Wau to Bulldog, south of Wau, along the central range of mountains. In each place there still exist large sections of undisturbed forest. The third area, for comparison, consists of secondary forest or grassland near Wau. Certain species of mammals occur in all three foci, viz., bandicoots, tree possums, Uromys tree rats, the murine Leptomys, and Rattus veracundus, a native forest rat that prefers the margins of the forest. There were significant differences in the distribution of some of the other murids. While Melomys were found in all three areas, the several species examined from the Adelberts were not taken in the other two areas, and vice versa. Rattus niobe, another native species, was the dominant rat in the high moss forests at Wau and Bulldog (about 2000m.), but is apparently absent in the Adelberts (perhaps because the maximum height of the mountains there is equivalent only to the lowest level of the range of this rat). Rattus ruber, a rat that was common in the grass and secondary vegetation at Wau and Bulldog, was rare in the Adelberts and was taken only at the very edge of the forest, and then only seldom. Rattus exulans, an introduced peridomestic rat found in grasslands and open secondary vegetation, was collected at Wau.

3. Of these various murines, only R. exulans and R. ruber belong in the category of "wild Rattus rattus" which we feel are so deeply involved in the ecology of chigger-borne rickettsiosis. If our theories are correct, then in New Guinea, as elsewhere, the vector-group of chiggers, namely Leptotrombidium (Leptotrombidium), should be restricted to those two Rattus, and to the secondary forest near Wau, rather than the relatively undisturbed forest in the Adelberts and along Bulldog Road. Such has proven to be the case, and the latter two areas have a trombiculid fauna that differs fundamentally from that found in scrub terrain. Even though the Contract has terminated, we are attempting to obtain additional data along these lines, using other funds, and plan to publish the results.

B. TOPOGRAPHY AND THE ENDEMICITY OF CHIGGER-BORNE RICKETTSIOSIS.

1. During World War II (5,6), and until our Army--sponsored investigations in Pakistan proved otherwise (26*, 24*, 29**, 30**, 34**, 35*) it was firmly believed that a glance at the terrain in question, or even an aerial photograph thereof, would serve to immediately determine whether scrub typhus was endemic in any particular Asiatic-Pacific area. This feeling was based upon the "characteristic" association of endemicity with secondary vegetation. However, as summarized by us in 1972 (34**), our studies proved otherwise, as shown by the following extracts:

C.L. WISSEMAN JR., M.D., PRINC. INVEST.: DADA17-70-C-0047 "TROMBICULID MITES (CHIGGERS) AND OTHER
ECTOPARASITES AS VECTORS OF RICKETTSIAL INFECTION," (1 SEPT. 1969-31 DEC. 1975) FINAL REPORT

"(1) Despite fundamental physiognomic and ecological differences and separation by many miles, two areas may harbor virtually the same fauna of small animals and ectoparasites, e.g. the mountain-deserts of Gilgit and the coniferous forests of the Kaghan Valley.

(2) In contrast, significant faunal differences, independent of altitudes, may occur within different parts of a region that appears essentially homogenous, e.g. the temperate forests near the mouth of the Kaghan Valley and those 65-110 km further north.

(3) 'Ecological islands' containing faunal and floral elements identical to those of the mesic Kaghan Valley exist in scattered mountainous areas amidst the deserts, of W. Pakistan, e.g., in Swat, Dir and Gilgit, and it is believed that similar refuges occur in neighboring parts of the USSR, Afghanistan, Nepal, Sinkiang, Tibet, etc. Biological barriers, such as vast stretches of desert or semidesert, perpetual icy peaks of mountain massif and broad rivers separate the areas in Pakistan, and hence it seems that the progenitors of these mammals and ectoparasites must have been present there before the uplift of the main Himalayas and the creation of xeric habitats in the Pleistocene and Recent epochs.

(4) Scrub typhus and tick typhus infections are present in all of these types of mountainous habitats, i.e., as "oasis scrub typhus" in the mountain-deserts of Gilgit, as well as in alpine and subarctic habitats in the mesic mountains. The relict faunas on the ecological islands may therefore be infected with other microbial agents associated with these same mammals and ectoparasites elsewhere, i.e., viruses of hemorrhagic fever, tularemia, etc.

2. In 1968 we reported another reason why topography and ecology alone are insufficient to determine endemicity (27*), namely that there is a notable time-factor, and hence with the passage of 5-10 years, terrain that superficially has remained unchanged in appearance may have varied from a hyperendemic focus of scrub typhus to one that is of low endemicity. What happens is that there are cyclical changes in the rats and vector species of trombiculids replaced by others, and these in turn may give way to different species in a few years (30**). As we stressed in 1972, (29**) this phenomenon has not yet been adequately appreciated, and hence misleading or erroneous reports have appeared in the literature, as when one group of workers report that their predecessors erred in saying a certain species of chigger or rat was predominant in a particular focus, or when investigators compare the endemicity of a focus today with one for an area that was reported 10 years ago, not realizing that the latter may be currently very different. It no doubt was this time-factor that accounted for the dearth of vector-Leptotrombidium in the classical foci in the gold-fields of New Guinea and their abundance in new areas there, where the presence of scrub typhus had never been previously suspected (22*).

We have been assembling data on these points as to how topography alone cannot be used to determine endemicity with chigger-borne rickettsiosis and plan to publish an article thereon.

P. OTHER STUDIES ON CHIGGER-BORNE RICKETTSIOSIS

With the partial support of this Contract, we organized a Colloquium on the zoogeography and ecology of ectoparasites, their hosts and related infections at the Second International Congress of Parasitology, Washington, D.C. (1970). Included in the Colloquium, and published in 1972 with our sponsorship and auspices as Editors (23**) were the following articles on scrub typhus: Traub et al (34**), Traub and Wisseman (29**) Boise (7**); Asanuma et al (4#) Kitaoka et al (9#); and Kitaoka (10#). Another pertinent publication was the chapter we prepared on scrub typhus (36*) in a standard text on tropical medicine.

C.L. WISSEMAN, JR., M.D., PRINC. INVEST: DADA17-70-C-0047 "TROMBICULID MITES (CHIGGERS) AND OTHER ECOTOPARASITES AS VECTORS OF RICKETTSIAL INFECTION," (1 SEPT. 1969-31 DEC. 1975) FINAL REPORT

G. HISTOLOGICAL STUDIES ON CHIGGER ATTACHMENT.

A preliminary report on the host reactions at the site of attachment of the chigger was published in 1972 (7**). Therein it was pointed out that the cellular environment (e.g. macrophages at this site may be of consequence in the transmission of R. tsutsugamushi both to and from the trombiculid mite. A paper on the details is in preparation.

III. TECHNIQUES OF REARING CHIGGERS.

A. After the Contract funds were terminated for the support of studies on the transmission of R. tsutsugamushi, we managed to utilize other monies to keep the most valuable of those unique colonies going for more than a year, until such time as the Walter Reed Army Institute of Research (at Forest Glen) was ready to receive and maintain them. These were the first mass cultures of chiggers ever established, since the methods for colonizing chiggers in other laboratories were based wholly upon the use of small containers. In conjunction with the Army scientists, we are writing a paper on the methods.

IV. TAXONOMY

A. In a revision of Helenicula of the Old World, we dealt with 28 species, including nine which were new to Science (14**). These chiggers are one of the major groups infesting Rattus and hence they are of especial interest. Diagnoses and illustrations were presented for all the species, along with a key for their identification. In the discussion on epidemiology in this article, it was pointed out that birds may be important in transporting these and other chiggers of rats over vast distances, e.g. Nepal-Pakistan-and Africa.

B. The genus Guntherana is one of the major taxa of trombiculids on rats in New Guinea, and new species are still being collected. We described three such (13**) and have more on hand. Additional New Guinean chiggers were also described in another collaborative project (11*). Because of the military importance of scrub typhus in Vietnam and the need for Army entomologists to know which species of chiggers the troops were encountering, we prepared a Report on the chiggers known to occur in Vietnam. Written in collaboration with M. Natchatram of the Institute for Medical Research, Kuala Lumpur, this Report was made available to the Army in unfinished form because of the urgent need for the document. It included diagnoses, keys for the identification of genera and species, illustrations of cardinal features, notes on hosts and habitats, etc., and served as the basis for the 1974 paper by Natchatram and Dohany (12*).

C.L. WISSEMAN, JR., M.D., PRINC. INVEST.:DADA17-70-G-0047 "TROMBICULID MITES (CHIGGERS) AND OTHER ECTOPARASITES AS VECTORS OF RICKETTSIAL INFECTION," (1 SEPT. 1969-31 DEC. 1975) FINAL REPORT

C. As the result of an ectoparasite survey we sponsored in Nepal, it was learned that of the 57 species of chiggers collected, 16 (28%) were Leptotrombidium, and of these 10 (62%) were new to Science (26*). Manuscripts are in preparation on this material, and descriptions are also being readied on the new taxa from Ethiopia. Under this Contract and its predecessors, a total of 62 new species of chiggers were described with the Principal Assistant as co-author, namely 12 and 50 respectively.

It is emphasized that no Contract funds were used for travel to Ethiopia, Nepal, New Guinea, etc. The opportunity for collecting there arose because of other projects, with mutually advantageous results.

V. OTHER RELEVANT STUDIES

A. Other publications by colleagues based upon the Army-sponsored field-work in Pakistan have appeared, e.g. two by Allred on mesostigmatid mites (1*, 2*), and two articles on mammal-hosts (17*, 18*).

BIBLIOGRAPHY AND REFERENCES CITED

- 1* ALLRED, D.M., 1969. New mesostigmatic mites from Pakistan with keys to genera and species. J.med.Ent. 6(3):219-244.
- 2* ALLRED, D.M., 1970. Two new mites (Laelaptidae) from W. Pakistan. J.med.Ent. 7(1):107-111.
- 3# ASANUMA, K., KITAOKA, M. & K. OKUBO, 1972. Endemic occurrence and possible vectors of Rickettsia orientalis in Nopporo, Hokkaido, the northern limit of geographic distribution of the agent in Japan. The Colloquium on the Zoogeography and Ecology of Ectoparasites, Their Hosts and Related Infections at the 2nd Internat. Congr. Parasit. (1970). J.med.Ent.
- 4# ASANUMA, K., KITAOKA, M. & K. OKUBO, 1972. Occurrence of scrub typhus in a classical endemic area in Japan not transmitted by Leptotrombidium akamushi. The Colloquium on the Zoogeography and Ecology of Ectoparasites, Their Hosts and Related Infections at the 2nd Internat. Congr. Parasit., Washington, D.C. (1970). J.med.Ent. 9(6):592.
- 5 AUDY, J.R., BOWER, J.D., HAWNING, H.C., BULLOCK, A.A., COCKINGS, K.L., FORD, W., GORDON, H. T.M., KALRA, S.L., PARKER, M.T., RADFORD, C.D., RONWAL, M.L. & H.M. THOMAS, 1947. Review of investigations and appendices. In: Scrub typhus investigations in South East Asia. War Office, Army Medical Directorate, London (Mimeo.) (Vide Trop. Dis. Bull., 1948, 45:62-70).
- 6 AUDY, J.R. & J.L. HARRISON, 1951. A review of investigations of mites typhus in Burma and Malaya, 1945-1950. Trans.Roy.Soc.Trop.Med.Hyg. 44:371-395.
- 7** BORSE, J.L., 1972. Tissue Reaction at the Site of Attachment of Chiggers. The Colloquium on the Zoogeography and Ecology of Ectoparasites, Their Hosts and Related Infections at the 2nd Internat. Congr. Parasit., Washington, D.C. (1970). J.med.Ent. 9(6):591.
- 8# KITAOKA, M., ASANUMA, K. & M. ISHIMARU, 1972. Seroprevalence of scrub typhus. The Colloquium on the Zoogeography and Ecology of Ectoparasites, Their Hosts and Related Infections at the 2nd Internat. Congr. Parasit., Wash. D.C. (1970). J.med.Ent. 9(6):594.
- 9# KITAOKA, M., ASANUMA, K. & J. OTSUJI, 1972. Trombiculid mites zoophilic for man in Akita Prefecture in the summer season. Loc. cit. J.med.Ent. 9(6):592.
- 10# KITAOKA, M., OKUBO, K., ASANUMA, K. & J. OTSUJI, 1972. Birds in the ecology of scrub typhus. Loc. cit. J.med.Ent. 9(6):594.
- 11* NADCHATRAM, M., 1970. A revision of intranasal chiggers with descriptions of 12 species from east New Guinea (Acarina, Trombiculidae). J.med.Ent. 7(1):1-29.
- 12* NADCHATRAM, M. & A.L. DOHANY, 1974. A pictorial key to the subfamilies, genera and subgenera of Southeast Asian chiggers (Acarina, Prostigmata, Trombiculidae). Bull.Inst.Med.Res., Malaysia No. 16:1-67.
- 13** NADCHATRAM, M. & R. TRAUB, 1969. Three new species of Guntherana Wormersley & Heaslip (Prostigmata: Trombiculidae) from East New Guinea. J.med.Ent. 6(3):268-272.
- 14** NADCHATRAM, M. & R. TRAUB, 1971. Chiggers of the genus Helenicula in the Old World. J.med. Ent. 8(5):562-597.
- 15# ROBERTSON, R.G. & C.L. WISSEMAN, JR., 1973. Tick-borne rickettsiae of the spotted fever group in West Pakistan. II. Serological classification of isolates from W. Pakistan and Thailand: evidence for two new species. Amer.J.Epid. 97(1):55-64.
- 16# ROBERTSON, R.G., C.L. WISSEMAN, JR. & R. TRAUB, 1970. Tick-borne rickettsiae of the spotted fever group in West Pakistan. I. Isolation of strains from ticks in different habitats. Amer.J.Epid. 92(6):382-394.
- 17* SCHLITZER, D.A. & A.F. DeBLASE, 1975. Taxonomy and geographic distribution of Rhinopoma microphyllum (Chiroptera: Rhinopomatidae) in Iran with the description of a new subspecies. Mammalia 38(4):657-665 (1974).

Footnote¹ For key to bibliographic citations see last page (12)

C.L. WISSEMAN, JR., M.D., PRINC. INVEST.: DADA17-70-C-0047 "TROMBICULID MITES (CHIGGERS) AND OTHER
ECTOPARASITES AS VECTORS OF RICKETTSIAL INFECTION," (1 SEPT. 1969-31 DEC. 1975) FINAL REPORT

- 18* SCHLITTER, D.A. & H.W. SETZER, 1973. New rodents (Mammalia: Cricetidae, Muridae) from Iran and Pakistan. *Proc. Biol. Soc. Wash.* 86(14):163-174.
- 19# TOYOKAWA, K., 1972. Transmission of *Rickettsia orientalis* from experimentally infected mice to vector mites. The Colloquium on the Zoogeography and Ecology of Ectoparasites, Their Hosts and Related Infections at the 2nd Internat. Congr. Parasit. (1970). *J. Med. Ent.* 9(6):593.
- 20* TRAUB, R., 1972. Notes on fleas and the ecology of plague. The Colloquium on the Zoogeography and Ecology of Ectoparasites, Their Hosts and Related Infections at the 2nd Internat.
- 21* TRAUB, R., 1972. The zoogeography of fleas (Siphonaptera) as supporting the theory of continental drift. *Loc. cit.* *J. Med. Ent.* 9(6):584-589.
- 22* TRAUB, R., BEG MIRZA, A., MADCHATRAM, M. & E.B. MANN, 1970. Survey of Bulolo for fleas and trombiculid mites. *Papua & New Guinea Med. J.* 13(2):62-64.
- 23** TRAUB, R. & B.L. ELISBERG (Eds.), 1972. The Colloquium on the Zoogeography and Ecology of Ectoparasites, Their Hosts and Related Infections at the 2nd Internat. Congr. Parasit. Washington, D.C. (1970). Nos. 1-32. *J. Med. Ent.* 9(6):583-605.
- 24# TRAUB, R. & T.M. EVANS, 1967. Description of new species of hystrichopsyllid fleas, with notes on arched pronotal combs, convergent evolution and zoogeography (Siphonaptera). *Pacific Insects* 9(4):603-677.
- 25# TRAUB, R. & M. MADCHATRAM, 1967. New species of chiggers of the subgenus *Leptotrombidium* from the mountains of West Pakistan (Acarina: Trombiculidae). *J. Med. Ent.* 4(1):1-11.
- 26# TRAUB, R. & M. MADCHATRAM, 1969. Appendix B - Entomological Report, pp. 123-155, in: Worth, R.M. & N.K. Shah: *Nepal Health Survey, 1965-1966*. University of Hawaii Press, 158 pp.
- 27# TRAUB, R. & C.L. WISSEMAN, JR., 1968. Ecological considerations in scrub typhus. 1. Emerging concepts. *Bull. W.H.O.* 39(2):209-218.
- 28# TRAUB, R. & C.L. WISSEMAN, JR., 1968. Ecological considerations in scrub typhus. 2. Vector-species. *Bull. W.H.O.* 39(2):219-230.
- 29** TRAUB, R. & C.L. WISSEMAN, JR., 1972. Diminution in the endemicity of scrub typhus with the passage of time. The Colloquium on the Zoogeography and Ecology of Ectoparasites, Their Hosts and Related Infections at the 2nd Internat. Congr. Parasit., Washington, D.C. (1970). *J. Med. Ent.* 9(6):590-591.
- 30** TRAUB, R. & C.L. WISSEMAN, JR., 1974. The ecology of chigger-borne rickettsiosis (scrub typhus). *J. Med. Ent.* 11(3):237-303.
- 31** TRAUB, R. & C.L. WISSEMAN, JR., 1974. Current concepts of the ecology of chigger-borne rickettsiosis (scrub typhus). *Jap. J. Med. Sci. Biol.* (Tokyo) 27(1):1-5.
- 32* TRAUB, R., WISSEMAN, C.L., JR. & A. FARHANG-AZAD, 1977. The Ecology of Chigger-borne Rickettsiosis and Murine Typhus - Changing Concepts and Epidemiology (Abstract). *Proc. 15th Int. Congr. Ent.* pp. 487-489.
- 33** TRAUB, R., WISSEMAN, C.L., JR., JONES, M.R. & J.J. O'KEEFE, 1975. The acquisition of *Rickettsia tsutsugamushi* by chiggers (trombiculid mites) during the feeding process.
- 34** TRAUB, R., WISSEMAN, C.L., JR., & M. MADCHATRAM, 1972. The zoogeography and ecology of scrub typhus. The Colloquium on the Zoogeography and Ecology of Ectoparasites, Their Hosts and Related Infections at the 2nd Internat. Congr. Parasit. (1970). *J. Med. Ent.* 9(6):589-590.
- 35# TRAUB, R., WISSEMAN, C.L., JR. & MUR AHMAD, 1967A. The occurrence of scrub typhus infection in unusual habitats in West Pakistan. *Trans. Roy. Soc. Trop. Med. Hyg.* 61(1):23-57.
- 36* WISSEMAN, C.L., JR. & R. TRAUB, 1976. Scrub Typhus (Chigger-borne Rickettsiosis). Chapter 12 (pp. 125-130). In: Hunter, G.W., III, Swartzwelder, J.J. & D.F. Clyde (Eds.) "A Manual of Tropical Medicine". 5th Edition, W.B. Saunders & Co., Philadelphia, 900 pp.

O.L. WISSEMAN, JR., M.D., PRINC. INVEST.:DADA17-70-C-0047 "TROMBICULID MITES (CHIGGERS) AND OTHER
ECTOPARASITES AS VECTORS OF RICKETTSIAL INFECTION," (SEPT. 1969-31 DEC. 1975) FINAL REPORT

FOOTNOTE¹ In the bibliographic citations in this Report, the designations after the reference number indicate the following:

- **** pertains to work specifically done under this Contract, and by the scientists listed herein.
- *** indicates studies accomplished with the partial support of the Contract, by us or our colleagues.
- #** refers to other articles published under the auspices of the Contract.
- @** signifies other Army-sponsored research, e.g. previous Contract, undertaken by us.

Unmarked citations refer to work by other investigators.

15

C.L. WISSEMAN, JR., M.D., PRINC. INVEST.: DADA17-G-0047 "TRONBICULID MITES (CHIGGERS) AND OTHER
BOTOPARASITES AS VECTORS OF RICKETTSIAL INFECTION," (1 SEPT. 1969-31 DEC. 1975) FINAL REPORT

PERSONNEL			
<u>NAME</u>	<u>% OF TIME</u>	<u>% OF SALARY % PAID UNDER CONTROL</u>	<u>DATES</u>
BORSE, J.	100%	NIL	July 1969-Aug. 1973
BRYAN, V.	80%	80%	(Throughout)
BRENNAN, L.G.	100%	100%	April-July 1970 only
FETTY, C.	100%	100%	Oct. 1971-Sept. 1973
LAWRENCE, J.	100%	100%	Nov. 1972-Sept. 1973
MALIKUL, P.	50%	50%	Paid only in Dec. 1971 and May 1972
O'KEEFE, J.J.	100% 50%	100% 50%	From onset in June 1969 to July 1973 July 1973-Nov. 1976
SHIPLEY, S.	100%	100%	June 1972-Sept. 1972 only
SMITH, S.R.	100%	100%	From onset through Apr. 1970.
TRAUB, R.	50%	20-25%	(Throughout)

12 Copies

Director (ATTN: SGRD-UWZ-AG)
Walter Reed Army Institute of
Research
Walter Reed Army Medical Center
Washington, D. C. 20012

4 Copies

HQDA (SGRD-SI)
Fort Detrick
Frederick, MD 21701

12 Copies

Defense Technical Information Center (DTIC)
ATTN: DTIC-DDA
Cameron Station
Alexandria, Virginia 22314

1 Copy

Dean
School of Medicine
Uniformed Services University
of the Health Sciences
4301 Jones Bridge Road
Bethesda, Maryland 20014

1 Copy

Superintendent
Academy of Health Sciences, US Army
ATTN: AHS-COM
Fort Sam Houston, Texas 78234